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IS 4011 (1997): Methods of test for safety evaluation of cosmetics [PCD 19: Cosmetics]



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भारतीय मानक
सौंदर्य प्रसाधन का सुरक्षा आकलन — परीक्षण विधि
(द्वितीय पुनरीक्षण)

Indian Standard
**METHODS OF TEST FOR
SAFETY EVALUATION OF COSMETICS**
(*Second Revision*)

First Reprint JULY 2008
(Including Amendment No. 1 & 2)

ICS 71.100.40

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BUREAU OF INDIAN STANDARDS
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NEW DELHI 110002

April 1997

Price Group 4

FOREWORD

This Indian Standard (Second Revision) was adopted by the Bureau of Indian Standards after the draft finalized by the Cosmetics Sectional Committee had been approved by the Petroleum, Coal and Related Products Division Council.

Cosmetics are used almost universally on a regular basis. Safety in cosmetics and toiletries is of prime importance, as only safe products should be allowed to be used by consumers. This Indian Standard was initially published in 1967 and first revised in 1982 and covered the methods for dermatological tests only. Subsequently the committee responsible for the preparation of the standard felt further revision was needed to enlarge the scope of the standard to cover the methods of test for safety evaluation of cosmetics and fall in line with the international trends in cosmetics safety testing with minimal use of animals without any compromise on safety of the consumer.

All cosmetic products should be formulated conforming to the restrictions imposed by IS 4707 (Part 1 and 2) and the lists of CTFA (Cosmetics, Toiletries and Fragrance Association, USA), EEC (European Economic Community) and the guidelines of IFRA (International Fragrance Association) as updated from time to time. Cosmetic products formulated on the above lines are likely to be safe and such products may not warrant any safety testing. However, any cosmetic preparation formulated using the ingredients indicated above, may be subjected to safety evaluation using animals, as stipulated in this standard, if the manufacturer feels it necessary. Any formulation not tested on animals may bear the label "The product has not been tested on animals for safety". Whereas products which contain novel ingredients which are not under the purview of the above documents would require safety testing using the guidelines provided in this standard.

In this revision, the standard has been divided into two sections. Section 1 provides guidelines for safety testing of cosmetics and section 2 covers general information on contact hypersensitivity and photo sensitization. It includes tests to enable the diagnosis of contact allergy and photosensitization in the event of a problem in the market place.

Depending upon the effect of various chemicals on the skin, these may be classified into the following four categories:

- a) *Cauterizing agents* — Such as strong acid or alkalis which will char the skin of almost every individual exposed to the agent;
- b) *Primary irritants* — Such as croton oil which will produce dermatitis on the skin following the very first application;
- c) *Repeated-insult irritants* — Such agents would produce irritant dermatitis only after repeated application; and
- d) *Contact allergens* — Which include substances which are capable of producing adverse immunological response on the skin resulting in the manifestation of allergic cutaneous reaction (hypersensitivity) such as itching, erythema, scaling, papules, vesicles, bullae, etc.

Sometimes the same substance can act as a cauterizing agent, a primary irritant, a repeated insult irritant or a contact allergen depending upon the concentration of the substance in the finished product.

Adequate National/Regional facilities and expertise for safety testing of cosmetics are to be established for proper conduct of the tests. And the tests are recommended to be conducted as per GLP (Good Laboratory Practice) in order to make the result internationally acceptable. It is hoped that the formulation of this standard and the use of the methods prescribed therein would ensure good manufacturing practices and safety of the cosmetics in use.

In reporting the result of the test made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (revised)'.

**AMENDMENT NO. 2 NOVEMBER 2007
TO
IS 4011 : 1997 METHODS OF TEST FOR SAFETY
EVALUATION OF COSMETICS**

(Second Revision)

(Page 2, clause 4.3.1) — Substitute the following for the existing clause:

4.3.1 Skin Irritation Test

4.3.1.1 Principle

Irritants are substances that may damage the skin. The damage will depend upon the nature, concentration and duration of exposure. Irritation is manifested as inflammatory responses such as erythema (redness), oedema (swelling), vesiculation and finally to an intense suppurate reaction without the involvement of immune system. The irritation potential of a substance can be assessed in human patch test. This patch test is carried out on human volunteers in the manner given below.

4.3.1.2 Procedure

Apply the neat cosmetic product sample as such on the upper arm of human subjects, under occlusive patch for duration of 24 h. In case of rinse off products, rinse the treated sites with water to remove any residue. However, if the volunteer experiences unbearable discomfort with any of the patches, the volunteer is instructed to remove such patches any time prior to the targeted 24 h contact. Mark such sites with a blue/black marker to facilitate evaluation later. The volunteer is also requested to note down the signs and symptoms of the discomfort and the time of removal of the patch and hand it over to the investigator. Assess the skin reactions subjectively using the Draize scale, given in Table 1, 24 h after removal of the patches. Follow up the reactions if any, one week later to confirm recovery.

4.3.1.2.1 Human subjects

Select 24 healthy adult human subjects, preferably equal number of males and females who do not have any previous history of adverse skin conditions and are not under any medication likely to interfere with the results. Pregnant ladies and breast feeding mothers should be excluded. Explain the test procedure to volunteers and obtain a signed informed consent from each of them.

Amend No. 2 to IS 4011 : 1997

4.3.1.2.2 Test patches for topical treatment

Ideally use ready-made standard test patches (Finn Chambers) measuring about 1 cm diameter. Fix three such test patches on a transparent porous surgical adhesive tape of sufficient length (approximately 14 cm) and breadth. Take 0.04 ml of the sample using a micropipette on the patch and apply the patch on the upper arm as mentioned in 4.3.1.2. Alternatively if such patches are not available, use 1 cm diameter discs made out of chromatography paper (Whatman No. 3) taken on a slightly bigger polythene sheet having about 0.25 cm hole punched at the centre and fixed on the adhesive tape. Keep about 2.5 cm distance between the two adjacent test patches (filter paper discs).

4.3.1.2.3 Positive control

Use sodium lauryl sulphate (SLS), analytical grade, at 3 percent (w/w) concentration in distilled water as the positive control.

4.3.1.3 Observation and scoring

Assess the skin reaction under a constant artificial daylight source, 24 h after the removal of the patches. Score the reactions, namely, erythema (including dryness, scaliness and wrinkles) on a 0-4 point scale and oedema on another 0-4 point scale as per the Draize Scale given in Table 1.

Table 1 Draize Scale for Scoring the Treatment Sites

(Clause 4.3.1.3)

Score for Erythema/ Dryness/ Wrinkles (1)	Reaction (2)	Score for Oedema (3)	Reaction (4)
0	No reaction	0	No reaction
1	Very slight erythema/dryness with shiny appearance	1	Very slight oedema
2	Slight erythema/dryness/ wrinkles	2	Slight oedema
3	Moderate erythema/dryness/ wrinkles	3	Moderate oedema
4	Severe erythema/wrinkles/ scales	4	Severe oedema

4.3.1.4 Result

The combined mean scores and standard deviation of the 24 subjects are calculated:

- a) Positive control must give a combined score of greater than 4. If it is less than 4, then the test need to be repeated on another group of newly recruited volunteers.
- b) A combined mean score of 2.0/8.0 will mean that product is non-irritant.
- c) Usage of cosmetic product with a score up to 4.0/8.0 which is mildly irritating may be reviewed by manufacturer for safety of the formulation.
- d) No cosmetic product should be marketed which has irritation score above 4.0/8.0.

[Page 3, clause **4.3.4.1 Eye Irritancy Test (Draize Test in Rabbits)**] --
Delete.

NOTE

1 Draze test is not permitted by Government of India.

**AMENDMENT NO. 1 MARCH 2002
TO
IS 4011 : 1997 METHODS OF TEST FOR SAFETY
EVALUATION OF COSMETICS**

(Second Revision)

(Foreword, para 3, line 7) — Delete the following sentence:

Any formulation not tested on animals may bear the label 'The product has not been tested on animals for safety.'

(PCD 19)

Indian Standard

METHODS OF TEST FOR SAFETY EVALUATION OF COSMETICS

(*Second Revision*)

1 SCOPE

This standard covers methods of test for safety evaluation of cosmetics.

2 NORMATIVE REFERENCES

The following Indian Standards contain provision which, through reference in this text, constitute provisions of this standard. At the time of publication the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below:

<i>IS No.</i>	<i>Title</i>
11601 (Part 2) : 1992	Synthetic detergents — safety evaluation — methods: Part 2 Method of test for skin sensitization potential of synthetic detergents (guinea pig maximization test)
13424 : 1992	Safety evaluation of bathing bars and toilet soaps — methods of test

3 TERMINOLOGY

For the purpose of this standard, the following definition and explanations shall apply.

3.1 Allergic Reactions

Symptoms/signs caused by exposure to allergens.

3.2 Bullae

Bullae are blisters on the skin each of which is more than 5 mm in diameter. These may be of any colour and shape.

3.3 Cauterizing Agents

Chemical substances which burn the skin on local application.

3.4 Cauterizing Reaction

The changes in the skin produced by the local application of a cauterizing agent.

3.5 Cross Sensitization

Sensitization to a primary allergen spreading to one or more allergens which are of such closely similar chemical constitution to the primary allergen that the sensitized cells are unable to distinguish between them. This has to be distinguished from false cross sensitization which occurs when the same chemical substance is present in different products.

3.6 Dermatitis

Inflammation of the skin.

3.7 Epidermal Barrier

Epidermal barrier pertaining to the most superficial layer of the skin (stratum corneum) which allows only certain substances to enter the skin from outside.

3.8 Erythema

Redness of the skin due to dilation of the blood vessels.

3.9 Exudation

The discharge of fluids (serum or pus) on the diseased skin surface.

3.10 Fissuring

Cracks on the surface of the skin.

3.11 Hyperaemia

Increased blood circulation.

3.12 Hypersensitivity (Sensitization)

A process by which an individual develops the capability of reacting in an abnormal (allergic) manner to an external agent.

3.13 Miliaria

A papular or vesicular eruption on the skin which accompanies profuse sweating and is due to the blockage of the ducts of the sweat glands.

3.14 Necrosis

Death of a portion of a tissue.

3.15 Occlusion Area

An area of skin which is isolated from the environment.

3.16 Papules

A small circumscribed solid elevation of the skin not larger than 5 mm in diameter. It may be of any shape and colour.

3.17 Percutaneous Absorption

Transfer of an agent (usually a chemical) through the skin from outside.

3.18 Photopatch Test

A test where the skin is exposed to sunlight or any other equivalent source of light after contact with a particular chemical agent.

3.19 Pustular Reaction

A reaction in the skin characterized by appearance of several lesions (eruptions) containing pus.

3.20 Repeated-insult Irritant

An agent which causes an irritant reaction only after repeated applications to the surface of the skin.

SECTION 1 SAFETY TESTS

4 GENERAL

4.1 Raw Material Purity

The purity of raw materials used in a formulation must be established by physicochemical analysis and specifications established for use, before the products containing them are subjected to safety testing.

4.2 Facilities

The testing should be carried out by the manufacturer in-house or in reputed laboratories both national and international, which maintain high standards such as compliance to GLP required for safety testing.

The housing, environmental conditions-relative humidity, lighting, temperature, clean filtered air, feed, water, etc, are prescribed in IS 13424.

The tests should be carried out by trained personnel under the supervision of toxicologists (for example qualified biochemists, zoologists, pharmacologists, veterinary or medical scientists with experience in this discipline).

4.3 Tests

The type of tests to be performed for particular type of cosmetics are given in Table 1.

Table 1 Tests to be Carried Out on Different Types of Cosmetics

Sl No.	Test	Type of Product
i)	Skin irritation test	Skin and hair products and lip products.
ii)	Oral toxicity limit test	Dentifrice, mouth wash and lip products.
iii)	Mucous membrane irritancy test:	
a)	Eye irritancy test and mucous membrane irritancy test*	Hair care products and eye area cosmetics
b)	Oral mucous irritancy test	Dentifrice, and mouthwash

*Currently this test is not allowed to be carried out as per *The Prevention of Cruelty to Animals Act 1960* and the rules therein.

Additional data may be generated on skin sensitization, if the product contains any suspect ingredient which are above the recommended levels that are indicated by the international agencies (see foreword).

Carcinogenicity testing may not be necessary. Human carcinogens identified by IARC monograph 1992 (and updated later) must never be directly added into the cosmetic formulations. Naturally occurring chemicals also need to be safety tested.

4.3.1 Skin Irritation Test (Draize Test in Rabbits)

4.3.1.1 Principle

Irritants are substances that damage the skin by direct toxic action. The damage will depend upon the nature of the irritant, its concentration and duration of exposure. Irritation is manifested as inflammatory responses such as erythema (redness), oedema (swelling), vesiculation and finally to an intense suppurate reaction without the involvement of immune system. The Draize 24-hour patch test in rabbits is the most widely used animal test for primary irritants.

4.3.1.2 Procedure

The method utilizes a patch test technique on the intact skin of the albino rabbit. A set of 6 rabbits is used for testing each material. The hair is clipped from the back and flanks at least 1 day prior to application of the product. Animals should be without any skin blemishes and the hair on the treatment sites should not be in the active phase of growth (anagen) so that it will not impede the observation of irritant manifestation. 4 areas of the back spaced approximately 2-3 cm apart are designated for the position of the patches and 3 products and 1 positive control can be simultaneously tested on one animal. Patch measuring approximately 10 cm² consisting of 12 ply surgical gauze is placed on a slightly smaller thin polythene sheet which in turn is placed on a suitable surgical adhesive plaster

tape. The material to be tested is introduced on the surgical gauze - 0.5 ml (in case of liquids) or 0.5 g in case of solid or semisolid. Solids should be moistened with distilled water/suitable vehicle before application. Each test material should be placed on a preselected area. Using sodium lauryl sulphate at different concentrations (say 2 to 20 percent - approximately 5 percent for 24 h patch and 20 percent for 4 h patch on active ingredient basis) identify before - hand a particular concentration which will give a score greater than 5. The concentrations thus identified should be applied as a positive control on each animal in an identical fashion as the test product. Holding the patches in their respective sites is reinforced by wrapping the whole trunk with a suitable cloth/ canvas bandage.

Products which come in contact with the skin only for a short duration such as shampoos should be tested only for a short duration say not more than 4 h and since such products are diluted before use, a test concentration of 8-10 percent is recommended.

4.3.1.3 Observation

The patches are removed after 24 hours and the skin sites are scored then (0 h) and 48 h later using the following scale:

Erythema and Eschar Formation

No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Total possible erythema score	4

Oedema

No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approx 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4
Total possible oedema score	4

The total erythema/eschar and oedema score for each site at 0 and 48 h are added and averaged from the 6 animals to give the primary irritation index (p.i.1) that is the total score of 6 animals (a) divided by total number of animals (6) multiplied by number of observations (2) that is $[a/12]$.

4.3.1.4 Conclusion

On the basis of the P.1.1 (see 4.3.1.3) the products could be classified as mild irritant (score up to 2) moderate irritant (score > 2 to 5) and severe irritant (score > 5).

Under the conditions of the test the positive control should produce a primary irritation score of 5 or more.

Cosmetic products should not produce moderate irritancy response.

Alternatively the human patch test described in IS 13424 may be carried out.

4.3.2 Skin sensitization Test (Mangusson and Kligman Guinea Pig Maximization Test)

The principle and test methodology are given in IS 11601 (Part 2).

Alternatively the Buehler test described in IS 13424 may be carried out.

4.3.3 Oral Toxicity Limit Test (Rats)

4.3.3.1 Principle

Oral toxicity bioassay is mainly intended to confirm that the product does not have any overt acute toxicity potential even under certain misuse.

4.3.3.2 Method of Test

The test shall be carried out on albino rats weighing about 100-120 g. Six animals (preferably equal number of males and females) are selected and starved overnight. The animals are then administered the cosmetic product at the highest possible concentration as solution/suspension/emulsion by gavage (stomach intubation) at a dose of 5 ml/100 g body weight. All the animals are given feed and water ad libitum after the administration.

4.3.3.3 Observation and Conclusion

The animals are observed daily for one week after intubation for any abnormality and mortality. Cosmetics which produce $LD_{50} 5 \pm 1$ g/kg body weight should be viewed with caution.

4.3.4 Mucous Membrane Irritancy Tests

4.3.4.1 Eye Irritancy Test (Draize Test in Rabbits)

4.3.4.1.1 Principle

Eye irritancy potential of a substance is evaluated on the basis of its ability to cause injury to the cornea, iris and conjunctivae when the substance is applied to the eye.

4.3.4.1.2 Procedure

The test is carried out on adult albino rabbits (weighing about 1.5 - 2 kg). 0.1 ml of 1 percent

solution of the substance is instilled into one of the eyes while the other eye is kept untreated to serve as control. A series of 6 animals are used for testing one substance. In 3 animals the treated eyes are washed with 20 ml of luke warm water (at body temperature) 2 seconds after the instillation. In the second group, the treated eyes are washed with a similar amount of luke warm water 4 seconds after instillation of the test substance.

4.3.4.1.3 Observation

Ocular reactions are read with the unaided eye. Readings are made at 24, 48 and 72 hours and at 4 and 7 days after treatment or as long as injury persists.

A preparation which elicits corneal and iris lesions which have not cleared by the seventh day is considered as a severe eye irritant.

4.3.4.1.4 Scoring

The cornea is scored on the basis of density of opacity and total area involved. The iris is scored on the intensity or degree of inflammation exhibited, and the conjunctiva is scored on the extent of chemosis, redness and discharge. The Draize scale for scoring ocular lesions is given below:

Scale of Scoring Ocular Lesion (Draize Method)

1) Cornea

A) Opacity-degree of density (area most dense taken for reading)

No Opacity	0
Scatter or diffuse area, details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris invisible	4

B) Area of cornea involved

One quarter (or less) but not zero	1
Greater than one quarter but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4
Scores A × B × 5	Total maximum = 80

2) IRIS

A) Values

Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these	1

or combinations of any thereof) iris still reacting to light (sluggish reaction is positive)

No reaction to light, haemorrhage, gross destruction (any or all of these). 2

Scores A × 5

Total maximum = 10

3) Conjunctivae

A) Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3

B) Chemosis

No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2
Swelling with lid about half closed	3
Swelling with lid about half closed to completely closed	4

C) Discharge

No discharge	0
Any amount different from normal (does not include small amount observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3
Score (A + B + C) × 2	Total maximum = 20

The total possible score would be 110. The critical score is about 30. Score exceeding 30 tend to represent irreversible damage. Scores less than 30 tend to clear away leaving no visible sign of damage.

The total score for the eye is the sums of all scores obtained for the cornea, iris and conjunctivae. A preparation eliciting more than mild reaction which lasts for more than 2 days on the ocular mucosa should not be considered safe as an eye area cosmetic.

Alternatively the test for the primary irritation of mucous membrane can be carried out using the guidelines given in IS 13424.

4.3.4.2 Oral Mucosal Irritation Test in Rats

4.3.4.2.1 Principle

Evaluation of oral mucosal irritation potential of products like dentifrice, mouth wash which come into contact with oral mucosa is necessary.

4.3.4.2.2 Procedure

The method employed involves daily treatment of the oral cavity of albino rats (6 per group) of 24-28 days old (body weight of about 35-40 g) with the product.

The animals are treated two times daily for four days. Each treatment consists of a 30 seconds swabbing of the interior of the mouth - say the pocket-like region between the inner lower lip (labial mucosa) and outer lower gum (incisor gingiva), the so-called lip-gum recess with 0.05 ml in case of tooth paste or 0.05 ml of a 50 percent aqueous slurry of tooth powder in distilled water or 25 percent solution of mouthwash in distilled water using an artbrush.

4.3.4.2.3 Observation

The oral mucosa of each animal is examined daily with a final examination three days following cessation of treatment. Irritation is scored as follows:

(A) Oral Mucosa

No reaction	0
Discoloration, slight sloughing	1
Sloughing in several areas	2
Ulceration	3

(B) Labial Junction

No reaction	0
Slight redness, sloughing, dryness	1
Skin rough and brittle, small sores	2
Cracking and bleeding	3

Irritancy scores are calculated by averaging the combined daily ratings for the oral mucosa and labial junction. Results are interpreted as follows:

- 0 - 0.4 Very mild
- 0.5 - 1.0 Mild
- 1.1 - 2.0 Moderate irritant
- 2.1 and above — Severe irritant

Dentifrice and mouth wash should not produce moderate irritancy response. It is desirable to include in this test an acceptable commercial formulations as a reference standard.

4.4 Use Test

4.4.1 After the cosmetic has passed the appropriate tests, 15 human volunteers shall be asked to use the cosmetic as normally used for 15 days. If there is no adverse reaction, the cosmetic may be released for consumer tests.

4.5 Restricted Consumer Test

4.5.1 The cosmetic may be then released into the market on a limited scale and should be closely

monitored for any adverse consumer response. It is customary to release between 5 000 and 10 000 units of the cosmetic. In case adverse reactions are reported/collected during this period, they should be studied adopting appropriate tests.

NOTES

1 It is important to realize that all tests are experimental laboratory based and therefore, the results cannot be considered to represent exactly what is likely to happen when the cosmetic is actually released for mass use. It will, therefore, be necessary to keep a watch on the reactions if any, which may occur following general release of the cosmetics when the agent is used by a much larger number of individuals and for much longer periods.

2 It is also known that there are some differences in the reactivity of the skin of various racial groups, the two sexes and various age groups. It will, therefore, be appropriate to perform the above mentioned tests on the same type of individuals who are targeted to be the main consumers of that particular cosmetics.

SECTION 2

5 GENERAL INFORMATION ABOUT CONTACT HYPERSENSITIVITY

5.1 Before an individual can manifest an allergic reaction to a locally applied agent, he normally develops hypersensitivity to the agent. In biological terms, this means that some lymphocytes in the blood acquire the capability of recognizing and reacting with the same chemical whenever and wherever that chemical is applied on the skin. An individual may start developing hypersensitivity following the very first exposure but generally it does not happen this way. Nevertheless, every exposure to the allergen has a chance of sensitizing the individual. Therefore, the more the number of exposures the greater the chances of developing hypersensitivity. This also means that an individual develops allergy to only those agents which he or she has already been using for several days, months, or even years. Once, however, an individual becomes allergic to a substance he is likely to develop a reaction following every subsequent exposure provided the exposure is adequate.

5.2 If an individual is exposed to a chemical agent for the first time, he is not likely to be allergic to that agent and, therefore, he cannot develop an allergic reaction. Sometimes, however, if the individual had already been allergic to another chemical agent, whose chemical structure resembles that of the present chemical, when even the so called first exposure will be able to produce an allergic reaction. This is called a reaction of cross-sensitivity. It is, however, important to be aware that many times, a person may get exposed to an agent without being aware of it. Thus he may become allergic during one such exposure and

develop an allergic reaction during the subsequent exposure.

5.3 Some substances may cause sensitization more easily than others. The chemical basis of this characteristic of these agents is not known, but such substances are considered to be more potent sensitizers than the others.

5.4 Startum corneum, the most superficial layer of skin is a very selective barrier which allows only some substances to pass through. It is quite obvious that if a substance cannot penetrate this barrier and enter the skin, it cannot cause contact allergy. In case, however, this barrier is defective due to disease or injury, there is a greater change of an allergic reaction.

5.5 The process of sensitization takes a minimum of 5 days during which there are no clinical signs or symptoms. Thus even if an individual starts developing hypersensitivity following the very first exposure, there will be no clinical signs or symptoms during the first 5 days. However, after the individual has become allergic, a subsequent exposure will result in signs and symptoms within a day or two.

6 CLINICAL SIGNS AND SYMPTOMS

6.1 The earliest indication of an allergic reaction is itching. This is soon followed by redness. If the reaction is mild, it may subside soon, but generally, because of continued exposures, the reaction progresses through papules, papulo-vesicles, vesicles and bullae, exudation and crusting. These reactions are called acute reactions. Subacute reactions generally result in erythema and scaling, while chronic reactions due to continued exposures may lead to darkening and thickening of the skin. On the lips and palms, the reaction frequently manifests as scaling and fissuring.

6.2 The reaction is generally limited to the area where the causative substance is applied. Sometimes, however, the agent may get applied on the other areas of the body by means of the fingers, clothes, pillows, etc. In such instances, the reaction will be seen at other areas as well.

6.3 If further applications of the agent are continued, the reaction keeps on increasing in severity and extent, but in case further applications are stopped, the reaction tends to subside even without any treatment.

6.4 The degree of allergy varies in different individuals. Some individuals are more severely allergic and, therefore, they will react to even smaller amounts of substance. Moreover, an allergic individual will develop a severe reaction if exposed to a larger amount of substance. Conversely, an aller-

gic individual may not develop any signs or symptoms if he is not exposed to an adequate amount of the substance.

6.5 Photocontact Dermatitis

Some agents, particularly dyes and perfumes, cause dermatitis only after the skin area on which they have been applied is exposed to sunlight or ultraviolet rays. Sunlight alone or the agent alone (without exposure to sunlight) does not result in dermatitis. This type of reaction often consists of darkening of the skin, though in acute cases, itching, papular, papulo-vesicular or scaly lesions may also be present.

7 DIAGNOSTIC TESTS FOR CONTACT HYPERSENSITIVITY

7.1 Patch Test

7.1.1 This test is based on the principle that in an allergic individual, the whole skin is capable of reacting with the allergen. Therefore, if the substance is applied on the skin on any part of the body, it will result in dermatitis at the test site. Although this test is simple, yet a little experience is essential for accurate interpretation of results.

7.1.2 The test should not be applied if the patient is already having acute dermatitis because there is a risk of aggravation of the dermatitis and occurrence of false positive reactions. The test should also be postponed if the patient is being given systemic corticosteroid or other immunosuppressive drugs, such as cyclophosphamide, methotrexate, azathioprine, etc, because these drugs can suppress the reaction and lead to false negative results.

7.1.3 The substance to be used as allergen for patch test may be either the finished product as such or preferably each of the suspect ingredient used in the product.

7.1.4 When using ingredients, it is important to use each agent in a specified concentration and also in a specified vehicle (or base). This is necessary to avoid false positive or false negative results, because if the concentration of the agent is too high, it may result in irritant dermatitis at the test site, while if the concentration is too low, it may not evoke any reaction. Similarly, if the vehicle used for dissolving the agent does not allow the agent to penetrate the epidermal barrier, it may result in a false negative reaction. In the case of cosmetic, it will be especially useful if agents which may be used as substitutes of the standard ingredients are also included for patch tests. This will help the industry to know the substitutes which may be employed in the manufacture of the cosmetic in place of the ingredient causing contact dermatitis.

7.1.5 Normal saline (0.9 percent aqueous solution of sodium chloride) or any other agent used as a vehicle for dissolving the ingredients, should also be applied as controls for the patch test. It is necessary to ensure that the controls do not produce any reaction at the site of patch test.

7.1.6 In some countries, ready-made patches are available for patch tests. In case they are not available, the same may be prepared as follows:

Cut out an approximately 4 cm square piece of adhesive plaster and fix on its center, a 2.5 cm square piece of cotton gauze. The gauze piece should be 4 to 8 layers thick. In the center of this gauze piece, place a small piece of cotton wool or a 1 cm square piece of thick filter paper. The gauze piece, cotton wool or the filter paper should be clean chemically.

7.1.7 The suspected allergen is smeared on or soaked into the central piece of cotton wool/filter paper. In case the substance is a liquid or an ointment, 0.05 ml of the same is sufficient. In case the substance is a solid, it should be powdered and wetted with 0.05 ml distilled water (for volatile substances, *see* 7.3).

7.1.8 Various allergens should be incorporated into different patches and all these patches should be applied on the skin of the patient so that the allergen is in direct contact with the skin. Various patches should be numbered and an accurate account of the allergens put on these patches should be entered into a register for records.

7.1.9 The patches are generally applied on the back, but in case the skin on the back is not suitable, the upper arm may also be used. The skin should essentially be non-hairy and free from any type of skin lesions. In case the back is hairy, the hair can be cut off by clipper or shaver before applying the patches.

7.2 Timing and Location of the Patch Test

7.2.1 The patches are left in place for approximately 48 hours. The individual is instructed to ensure that the patches do not get removed during this period and to avoid wetting them during bathing. After 48 hours the number of the patch should be marked on the adjoining skin and the patches removed. The sites where the allergens were applied on the skin should be encircled for identifications and the individual should be instructed to avoid scratching these areas. Half an hour after removal of the patches, the sites of the patch test should be inspected for evidence of dermatitis. Presence of dermatitis at any of the test sites indicate that the individual is allergic to the substance

applied at that site. The patch test reactions are generally graded as follows:

Description and Symbol	
No reaction	—
Erythema only	+
Erythema with papules	++
Papulo-vesicular reaction	+++
Ulceration or necrosis	++++

7.2.2 Reactions produced by solid substances are generally milder, as the amount of the allergen that can enter the skin is usually much less. Thus even milder reactions produced by solid agents should be considered significant.

7.2.3 Sometimes, when the hypersensitivity in a patient is very mild, there may be no reaction at the patch test site after 48 hours, though next day, there may be evidence of dermatitis. It is, therefore, preferable to take another reading after approximately 72 hours.

7.2.4 Sometimes, the patient shows evidence of dermatitis 5 days after application of the patch test. This indicates that the patient was not allergic to the chemical earlier, but the patch test has sensitized him.

7.2.5 Some individuals develop dermatitis reaction to the adhesive plaster which may spread to involve the entire test site, making it difficult to observe any reaction to the allergen. In such an instance the patch tests can be applied on the upper arm using only small strips of adhesive plaster or suitable hypo allergic plaster.

7.2.6 Sometimes particularly in summer, the patient may develop a miliaria (prickly heat) - like reaction at the patch test site due to occlusion. This reaction is not reproducible and, therefore, it should be regarded as false positive reaction.

7.2.7 Some chemicals particularly nickel sulphate can at times lead to a pustular reaction which also is non-specific.

7.3 Testing with Volatile Substances

7.3.1 Volatile substances can act as primary irritant if test by the standard occluded patch test technique. Such agents should, therefore, be tested by the cup technique. In this method, the volatile agent is soaked into a small piece of absorbent cotton filter paper which is placed at the bottom of specially designed (*see* Fig. 1). This cup is inverted on the forearm-skin of the individual and bandaged. Care should be taken to ensure that the piece of filter paper remains sticking to the bottom of the cup and does not fall on the skin, and the skin is exposed only to the vapors of the agent.

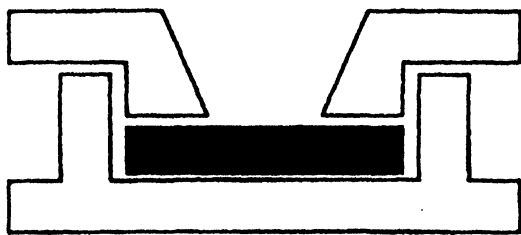


FIG. 1 ANTIGEN CUP

7.3.2 If a finished product containing volatile substances is to be tested, the agent should be painted on the skin and left exposed for 30 minutes to allow the volatile substance to evaporate. After this, the area is occluded as for the standards patch test.

7.4 Open Patch Test

7.4.1 Some substances such as shampoos, shaving creams, tooth pastes and other similar cosmetics may produce a glazed reaction by the occluded standard patch test which may be mistaken for a positive reaction. Such agents should preferably be tested by the open patch test method as follows.

7.4.2 The agent is applied on the skin and left as such for a minimum of 24 hours. The results are read 48 hours after the application as for the standard patch test.

NOTE — A report of a patch test is not complete unless it mentions the following data:

- a) Concentration of the chemical/agent;
- b) Amount used;
- c) Area of skin contacted;
- d) Site of application;
- e) Number of days and patch was left on the skin; and
- f) Period after removal of the patch that the readings were made.

7.5 Exposure and Withdrawal Test

7.5.1 Hypersensitivity of an individual to an agent can also be confirmed by asking the individual to

stop using the suspected agent for a week or so. If the reaction subsides during this period, it suggests that the agent was probably responsible for the dermatitis. Then the individual should be asked to use the agent again. In case the reaction reappears within the next 2-3 days, it is suggestive of the causal relationship of the dermatitis with the agent. For confirmation, withdrawal of the agent and exposure can be repeated. In case multiple agents are suspected, it will be necessary to re-expose to these agents one by one and to add every new substance after an interval of at least 2 or 3 days and preferably after 7 days.

7.6 Photopatch Test

7.6.1 In case a substance is considered to produce dermatitis by photocontact-sensitivity, it will be necessary to undertake photopatch tests rather than the standard patch tests. For this, each substance is applied in duplicate patches in the same manner as for standard patch tests, but after 24 hours, one of the patches in each pair is removed and the skin at the test site is exposed to sunlight for 30 minutes or an appropriate source of ultraviolet light. These patches are covered again. The other patches in each of the pairs is left undisturbed. One additional site in the adjoining skin is exposed to sunlight or ultraviolet light for the same period to act as a control for the ultraviolet exposure. After a further period of 24 hours, all the patches are removed and the test sites are examined for evidence of dermatitis. The photopatch test is considered to be positive if there is no reaction at the site of the standard patch test (not exposed to light), but the site of the exposed patch test shows evidence of dermatitis. The skin area taken as 'control' and exposed only to sunlight or ultraviolet light should also not develop any dermatitis.

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